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14. ABSTRACT

In this project, we have been developing a novel mouse model of multiple sclerosis with a focus on cognitive dysfunction associated with defective myelin. We have developed this model using homologous recombination in embryonic stem cells and have obtained homologous recombinants. However, we have experienced difficulties in deriving mice from the recombinant cells which has delayed our progress toward completion of our goals. We have now repeated the homologous recombinant experiments and are screening for embryonic stem cell clones to be used in homologous recombinant injections. In addition to generation of our novel model, we have been developing neurophysiological techniques to assess cognitive deficits in our novel mouse model. These experiments involve using the auditory pathway to test neural processing in the superior olivary complex, and are proceeding well.

15. SUBJECT TERMS
mouse model, multiple sclerosis, myelin, auditory pathway, cognition, homologous recombination, conditional knockout

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INTRODUCTION

The pathophysiology of demyelinating plaques in MS have been characterized in great detail and many of the underlying mechanisms causing physical disability in the relapsing/remitting and progressive phases of the disease have been elucidated. However, more subtle aspects of the disease which affect the day-to-day quality of life of patients have received much less attention, including cognitive and learning deficits, memory loss and difficulties with vision and hearing. This issues are of importance because they affect more than 50% of MS patients at all stages of the disease. Published clinical and case studies suggest that these symptoms stem from demyelinating/remyelinating lesions in white and gray matter regions, resulting in slower conduction velocity or intermittent conduction block through the lesions. If so, then cognitive and sensory deficits likely arise from structural abnormalities such as thin myelin, which has lower electrical resistance than normal and does not support rapid conduction velocities in small diameter axons. In this project, we will develop a knockout mouse with abnormalities in CNS myelin sheaths that mimic the electrical properties of myelin typically found in demyelinating/remyelinating MS plaques. The contributions of this dysfunctional myelin to hearing abnormalities and cognitive deficits will be determined using several electrophysiological tests.

BODY

Homologous recombination

We generated a DNA construct that inserts lox P sites into the *Claudin 11* gene in the 5' untranslated region and in intron 1 to flank the open reading frame in exon 1. We have previously generated a complete knockout of this region of the gene and have shown that neither functional mRNA nor protein are synthesized (Gow et al., 1999). The construct comprises 2 kb of *Claudin 11* genomic sequence upstream of exon 1 and 4 kb downstream. Electroporation of this construct into 129 embryonic stem cells yielded 60% recombination and we chose 12 clones for further characterization.

Several clones were eventually expanded, injected into mouse blastocysts and implanted into recipient mothers. Unfortunately, several of these clones either yielded chimeric mice with low levels of chimerism. Others resulted in high chimeras but technical problems led to loss of the mice before transport to Wayne State University. Finally, chimeras that were obtained did not breed to the germline. We have recently isolated several additional homologous recombinant clones and are currently characterizing them before they are injected into mouse blastocysts. Accordingly, this aspect of our work has proceeded slowly and has resulted in our applying for a no-cost extension to this grant.

Novel anesthesia protocol

In the past, we have recorded auditory brainstem responses (ABRs) from mice, which require approximately 30 min under avertin anesthesia (Gow et al., 2004). This drug has particularly useful characteristics because it is fast acting, is tolerated very well by the mice, maintains anesthesia for 40 min and does not significantly depress brainstem EEG activity. In the current project, we are assessing neural processing of auditory signals in the superior olivary complex of mice and we need to record electroencephalograms (EEGs) of mice for approximately 60 min under anesthesia.

To be able to record EEGs in the mice for an extended period, we developed a novel combination anesthesia protocol using avertin and chloral hydrate. Similar to avertin, chloral hydrate does not depress brainstem EEG activity and is well tolerated by mice. We tested several combinations of these anesthetics, alone or in combination, and determined that a combination dose of 375 mg/kg avertin:200 mg/kg chloral hydrate.

Neurophysiological protocol

In previous projects, we have determined that ABR measurements in *Claudin 11* knockout mice are abnormal in that the latency of Wave V is delayed by as much as 1 ms (Gow et al., 2004). Analogous changes also occur in the visual pathway (Gow et al., 1999). Such changes in latency in the brainstem, which we hypothesize will alter the temporal processing of auditory signals and perturb output from the neural circuitry in the superior olivary complex, arises because the myelinated fibers in the auditory brainstem are defective and

conduct more slowly (Devaux and Gow, 2008; Gow and Devaux, 2008). Because slowed conduction is a feature of small diameter myelinated axons in these animals, perturbed neural processing in the brainstem will be a model for other brain regions where such changes can affect affect behavior. In future we will measure behavioral changes in these mice and identify drugs that can reverse these abnormalities.

In the auditory field, measurement of auditory signals in the brainstem of mice is typically limited to ABRs and otoacoustic emissions. More sophisticates measures, such as neural processing of binaural responses are typically performed in rats, guinea pigs or larger mammals. Accordingly, we have been optimizing protocols in mice to take advantage of knockout technology in this species. Currently, we have developed a protocol to measure interaural level differences in our mice at 1 month of age and beyond. The protocol involves simultaneous stimulation of each ear of anesthetized mice using different sound intensities in each ear (e.g. 80 dB SPL in the left ear and 50 dB SPL in the right ear) and measurement of the resulting EEGs in the 12 ms following sound presentation. By subtraction of the EEGs that we obtain for the left and right ears we are able to calculate the binaural component of the EEGs for comparison of wild type and *Claudin 11* knockout responses. We are awaiting the derivation of our conditional knockout mice to begin these comparisons.

Brainstem neurotransmitter levels

In addition to neurophysiological changes in the superior olivary complex associated with the defective myelin in *Claudin 11* knockout mice, we anticipate that metabolic changes may be apparent. To this end, we have conducted an initial experiment by magnetic resonance spectroscopy (MRS) using an 11 Tesla magnet (500 MHz) to determine if we can observe changes in neurotransmitters and a number of other major metabolites. We have determined that levels of the neurotransmitter, glutamate and its metabolite glutamine, are increased in the superior olivary complex compared to controls, while other brain regions have normal levels. This region-specific change in an excitatory neurotransmitter signifies increased neural activity in the auditory pathway of the mutants which is likely a consequence of perturbed neural processing of auditory signals and confirms that abnormal myelin can have significant consequences for neurons and neurotransmitter systems.

KEY RESEARCH ACCOMPLISHMENTS

1. Homologous recombination in mouse embryonic stem cells to generate an inducible knockout of the *Claudin 11* gene.
2. Development of a novel anesthesia protocol to measure binaural auditory signals in the superior olivary complex of the mouse.
3. Development of a neurophysiological protocol to assess neural processing of binaural auditory signals in the superior olivary complex of the mouse.
4. Measurement of neurotransmitters and other neurochemicals in the superior olivary complex of *Claudin 11* knockout mice. Levels of glutamate and glutamine are increased in the superior olivary complex of the mutant mice.

REPORTABLE OUTCOMES

Abstracts:

1. Maheras K, Vengalil, M, Gow A (2011) Cognitive deficits associated with CNS myelin dysfunction. Great Lakes Glia, Traverse City, MI, Sept 24-26.
2. Maheras K, Gow A (2011) Cognitive deficits associated with CNS myelin dysfunction. Society for Neuroscience, Washington DC, Nov 12-16.
3. Denninger AR, Maheras K, Kirschner DA, Gow A (2011) Claudin 11 regulates the permeability of the CNS intramyelinic compartment. Society for Neuroscience, Washington DC, Nov 12-16.
4. Maheras K, Gow A (2011) Cognitive deficits associated with CNS myelin dysfunction. Am. Society for Cell Biol., Denver, CO, Dec 3-7.
5. Maheras K, Galloway M, Douglas MS, Ghoddoussi F, Gow A (2012) Cognitive Deficits Associated with Myelin Dysfunction and Neuronal Dyssynchrony. Society for Neuroscience, New Orleans, LA, Oct 13-17.

Informatics:

1. Inducible knockout of the *Claudin 11* gene in mice

Grant applications:

1. 1 F31 MH097469-01, NIH, NINDS (PI: K. Maheras) 4/1/12–3/31/14.
   Title: The role of Claudin 11 on neural processing and behavioral abnormalities
   Requested: $74,118.

2. 1 F31 MH097469-01A1, NIH, NINDS (PI: K. Maheras) 9/1/12–8/31/14.
   Title: The role of Claudin 11 on neural processing and behavioral abnormalities
   Requested: $84,464.

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1. K. Maheras, PhD graduate student, Wayne State University 6/31/11.
2. M. Vengalil, freshman year Princeton University, New Jersey 6/30/11–8/15/11.
   Summer Undergraduate Research Program, Wayne State University
3. K. Maheras, advance to PhD candidacy status, Wayne State University 1/31/12.

Personnel receiving pay from this award:

Alexander Gow
Kathleen Maheras

CONCLUSION

Although the specific goals of our research to generate a conditional knockout of the *Claudin 11* gene in mice and assess neural processing in the auditory pathway have been significantly delayed, we have made substantial progress and near completion for developing the necessary protocols to measure changes in behavior and cognition from the perspectives of neurophysiology and neurochemistry. We anticipate applying these techniques to the conditional knockout mice. In addition, we have been very active in collaborating with other groups to develop and analyze these mice in terms of published abstracts, student training and grant submissions.

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Gow, A., and Devaux, J.J. (2008). A model of tight junction function In CNS myelinated axons. Neuron Glia Biol 4, 307-317.

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